Bionomics of the established exotic mosquito species Aedes koreicus in Belgium, Europe

Versteirt, V ; De Clercq, E M ; Fonseca, D M ; Pecor, J ; Schaffner, Francis ; Coosemans, M ; Van Bortel, W

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Author(s): V. Versteirt, E. M. De Clercq, D. M. Fonseca, J. Pecor, F. Schaffner, M. Coosemans, and W. Van Bortel
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Sampling, Distribution, Dispersal

Bionomics of the Established Exotic Mosquito Species *Aedes koreicus* in Belgium, Europe

V. VERSTEIRT,[1,2] E. M. DE CLERCQ,[2] D. M. FONSECA,[3] J. PECOR,[4] F. SCHAFFNER,[2,5] M. COOSEMANS,[1,6] and W. VAN BORTEL[1]

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ABSTRACT Adults of an exotic mosquito, *Aedes (Finlaya) koreicus* (Edwards) (Diptera: Culicidae) were identified by morphology and genotyping from one site in Belgium in 2008. In late summer of that year, the occurrence of adults and immature stages reconfirmed its presence. This is the first record of this species outside its native range and in particular in Europe. Two subsites of the original location were prospected from April until October 2009 with different traps to evaluate the extent of its presence and establishment in the area and to understand the dynamics of the species' population. Next to *Ae. koreicus*, 15 other mosquito species were collected. Adult individuals of *Ae. koreicus* were found from May to September and larvae were still found early October. Larvae were mainly retrieved from artificial containers both in 2008 as in 2009. Containers with eggs and/or larvae were found up to 4 km away from the initial location, indicating the species is spreading locally. Though the introduction route is unknown, it may have occurred via international trade as a large industrial center was located nearby. A comparison of different climatic variables between locations in Belgium with *Ae. koreicus* and putative source locations in South Korea, revealed similarities between winter temperatures and the number of freezing days and nights in four consecutive years (2004–2008), while humidity and precipitation values differed strongly. The introduction of a new potential disease vector into Europe seems to be a result of proper entrance points, created by intense worldwide trade and suitable environmental conditions.

KEY WORDS *Aedes koreicus*, exotic species, establishment, population dynamics, climate

It is generally accepted that because of increasing speed and their pervasiveness, modern transport networks, global trade, transport, and tourism are becoming more and more pivotal in the spread of vectors and the infectious diseases they transmit (Wilson 1995, Wilson et al. 2009, Pysek et al. 2010). In southern Europe, past and recent importations of mosquito vector species such as *Aedes (Stegomyia) aegypti* (L.) and *Aedes (Stegomyia) albopictus* (Skuse) have created suitable conditions for local outbreaks of yellow fever, dengue, or chikungunya fever (Christophers 1960, Eritja et al. 2005, Angelini et al. 2007, Fontenille et al. 2007, La Ruche et al. 2010, Schmidt–Chanasit et al. 2010, Grandadam et al. 2011). Of all mosquitoes worldwide, aedines seem currently to have the highest invasive potential as the eggs of these species tolerate considerable periods of desiccation thus surviving long transports (Reiter and Sprenger 1987). *Aedes* species imported into central and northern Europe include *Ae. albopictus* (Schaffner et al. 2004), *Aedes (Finlaya) japonicus japonicus* (Theobald) (Schaffner et al. 2009, Versteirt et al. 2009), *Ae. aegypti* and *Aedes* (*Ochlerotatus*) *atropalpus* (Coquillett) (Scholte et al. 2009, 2010). J. F. Reinert (2000), divided the genus *Aedes* Meigen into genera *Aedes* and *Ochlerotatus* (Lynch Arribalizaga) on the basis of "consistent primary characters" and supplemental features. *Ochlerotatus* was elevated to generic rank and was further divided into two sections based on features of the fourth-instar larvae and pupae. In subsequent publications the genus *Aedes* was further dived and more subgenera were raised to genus level. However, the controversy surrounding this separation has left nontaxonomists in doubt. We have decided not to follow the taxonomy of Reinert (2000) and Reinert et al. (2009). Thus, global trade expansion is an important determinant in the worldwide introductions of mosquito species (Tatem et al. 2006). Yet, invasion success depends on a combination of interacting factors such as the suitability of the invaded habitat, the biological context and climatic

[1] Department of Parasitology, Entomology Unit, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium.
[2] Avia-GIS, Risschotlei 33, 2980 Zoersel, Belgium.
[3] Center for Vector Biology and Entomology Department, Rutgers University, 180 Jones Avenue, New Brunswick, NJ 08901.
[4] Walter Reed Biosystematics Unit, Museum Support Centre, MRC-534, Smithsonian Institution 4210 Silver Hill Road, Suitland, MD 20746-2863.
[5] Institute of Parasitology, University of Zürich, Winterthurerstrasse 266a, CH-8057 Zürich, Switzerland.
[6] Department of Biomedical Sciences, Faculty of Pharmaceutical, Veterinary and Biomedical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen (Wilrijk), Belgium.

similarity between source and invaded region (Kovats et al. 2001, Wilson et al. 2009). Once all conditions for the integration into a local environmental setting are fulfilled and a self-maintaining population can establish, an increase in density and range of the species will occur. Some can remain local for several years (exhibiting a long lag phase) after introduction; however, it is expected that all may become invasive at a given moment in time (Crooks and Rilov 2009, Hobbs and Humphries 1995). Combinations of similar environmental conditions are important to the establishment and spread of an invading disease vector and thus consequently facilitate the possibility of emergence of vector-borne diseases (Randolph and Rogers 2010).

A national mosquito inventory in Belgium (www.modirisk.be) revealed the presence in Belgium in 2008 of a new exotic species *Aedes (Finlaya) koreicus* (Edwards) before not known to spread beyond its original area (South Korea, Japan and parts of China and USSR) (Versteirt et al. 2012). The species is closely related to members of the *Ae. japonicus* complex, displaying overlapping characteristics and strong molecular relationship (Tanaka et al. 1979, Cameron et al. 2010). *Aedes j. japonicus* and *Ae. koreicus* are sympatric both in mainland Korea and in Jeju-do island (Tanaka et al. 1979, Lee 1994) yet they exhibit some behavioral differences. *Ae. koreicus* seems better adapted to urban environments than the forest dwelling *Ae. j. japonicus* that is surprising given the recent worldwide invasions of the latter species. Probably this was merely by chance and there is a distinct possibility that *Ae. koreicus* will also spread worldwide (Cameron et al. 2010).

Notwithstanding the fact that there is little information on *Ae. koreicus*, some data are available on its possible vector role from its origin region. In parts of Russia the species is suspected to be a vector for Japanese encephalitis (Miles 1964, Shestakov and Mikheeva 1966) but this was never reported from field observations in Korea (Kim et al. 2009). Feng (1938) reported that a population of *Ae. koreicus* from Peiping has been experimentally proven to be an efficient transmitter of *Dirofilaria immitis* to dogs. A recent report of the Korean Centres for Disease Control and Prevention (KCDC 2007) states that this species has intermediate vectorial capacity for the transmission of *Brugia malayi* to humans. Yamada (1927) found that it can pick up microfilariae but does not allow the development of *Wuchereria bancrofti.* These pathogens are not known to circulate in Belgium and there is currently no indication of any local vector role. Thus, the actual vectorial capacity of the species remains uncertain.

The aim of this study was to assess the introduction of *Ae. koreicus* from Jeju-do, the assumed origin region based on their morphological similarities (Versteirt et al. 2012) and its acclimatization in Belgium, to elucidate the establishment of the species in the area and to explore its population dynamics.

Materials and Methods

Mosquito Collections. The Mosquito Magnet Liberty Plus trap (MMLP, Woodstream Corporation, Lititz, PA) was used throughout Belgium during the MODIRISK mosquito inventory of 2007 and 2008 in a grid-based sampling approach in which different habitats in each grid were randomly sampled. One of those ≈1,000 randomly chosen sites was an old sand quarry near the national park Hoge Kempen and the industrial park of Maasmechelen, which harbors several recycling companies. One trap was placed in a small mixed forest fragment with birch, oak and pine next to the industrial zone (50.9959° N, 5.6209° E, site 1) and was operational for seven consecutive days from 20 to 27 May 2008. A second trap was subsequently operated from 23 to 30 September 2008 on the opposite side of the main road in a similar environment (50.9941° N, 5.6182° E, site 2).

To assess the degree of establishment and population dynamics of *Ae. koreicus*, a longitudinal study was conducted in 2009. Adults were collected with different trap types on both sites: two Mosquito Magnet Liberty Plus traps, two BG Sentinel traps (Biogents AG, Germany) and two Center for Disease Control and Prevention (CDC) Gravid traps (Frommer Updraft, John W. Hock company, Gainesville, FL). The first two trap types were placed in a Latin Square pattern, the latter in the prolongation of one MMLP and one BG Sentinel trap. All were positioned at least 68 m apart, to avoid signal interference. All traps operated fortnightly for 48 h starting from 20 April until 19 October 2009.

A preliminary random screening of potential larval development sites was performed on different occasions between June and November 2008 in an area of ≈1.5 km^2 around the adult collection sites. An extensive inventory of possible oviposition sites was conducted in 2009 on both sites during three visits: once in spring (May), once during summer (Mid-June), and once during late summer (Mid-September). At each visit, ≈20–30 potential artificial and natural oviposition sites were screened. Artificial containers included abandoned water recipients and tires while natural sites comprised tree holes, puddles, ponds, and stagnant water in mud tracks. Sampling was focalized in the industrial zone of Maasmechelen (car recycling company, construction enterprise) and the surrounding natural environment (mixed forests, sand quarry, and heath area). All larvae found were collected using and 500 ml dippers, small sieves, and a pipette, and were transported alive to the laboratory in vials labeled with site specific identification details. Once in the laboratory, larvae were killed by a thermal shock with hot water (60°C) and stored in 80% ethanol. Treated larvae were then identified using a stereoscopic microscope and a computer-aided identification key of Schaffner et al. (2001).

To estimate the local dispersal of the species, 17 ovitraps (black 1 liter flower pots) with a polystirene float (5 × 5 cm) as oviposition support and filled with an infusion baited mixture (described by Scott et al.

Table 1. List of variables included in the climatic comparison between Belgium and Jeju-do

Variables (no. variables included)		Corresponding variables
No. days with temperatures < 0°C	2004, 2005, 2006, 2007, 2008 (5)	2004, 2005, 2006, 2007, 2008
No. nights with temperatures < 0°C	2004, 2005, 2006, 2007, 2008 (5)	2004, 2005, 2006, 2007, 2008
Relative humidity	Jan. to Dec. (12)	Oct.
Total precipitation (mm)	Jan. to Dec. (12)	None
Average monthly min. temp (°C)	Jan. to Dec. (12)	Mar., April, May, Oct., Nov.
Average monthly mean temp (°C)	Jan. to Dec. (12)	Jan., Feb., Mar., April, Nov., Dec.
Average monthly max temp (°C)	Jan. to Dec. (12)	Jan., Feb., Mar., Dec.

A climatic variable is considered similar if the values in Belgium fall within the range of values recorded at Jeju-do.

2001) were set up. They were placed in three paths in northern (characterized by mixed forest), eastern (characterized by heathland nature reserve), and southern (characterized by forest-urbanized area) direction up to 5 km from site 1. Oviposition supports were collected monthly (from May until October 2009) and brought back to the laboratory where they were placed in water filled recipients in small cages until larval eclosion. Later, fourth stage larvae were killed, stored, and identified according to the method described above.

All morphological identifications of adult mosquitoes were done according to Tanaka et al. (1979), Schaffner et al. (2001), and Becker et al. (2003). The rapid assay developed by Cameron et al. (2010) was used on extracted DNA of eggs, larvae and adults to molecularly support the morphological identification of *Ae. koreicus.*

Climatic Data Analysis. Different morphological variants of *Ae. koreicus* have been described (Tanaka et al. 1979) and the specimens observed in Belgium are very similar to the form occurring on the island of Jeju-do (Versteirt et al. 2012). Based on these similarities it was assumed that the Belgian specimens originated from Jeju-do. Therefore, for in total 70 climatic variables, divided over seven main categories (see Table 1), the minimum and the maximum value for Jeju-do were determined. Then we delineated the areas in Belgium where the values for the respective climatic variables fell within the value range in Jeju-do.

Relative humidity was modeled by a Random Forest approach (Breiman 2001), using a set of global weather station data and a number of remotely sensing images, such as elevation, temperature, and land cover. Monthly minimum, mean, and maximum temperatures (Tmin, Tmean, Tmax) as well as total monthly precipitation were derived from the Worldclim dataset (Hijmans et al. 2005). The number of freezing days and nights were derived from MODIS A2 images (United States Geological Survey [USGS] 2009). A 5-yr archive of weekly images of day and night Land Surface Temperature (LST) was downloaded (USSG, 2009) and gaps in the data were filled using a temporal spline interpolation (Scharlemann et al. 2008). Temporal spline interpolation was also used to create daily images of day and night temperature.

Results

Mosquito Collections and Identification. In 2008, a total of 132 adult mosquitoes belonging to 14 species were collected (Table 2) of which six were adult *Ae. koreicus* mosquitoes. In 2009, 69 individuals of *Ae. koreicus* were found out of a total of 1,950 individuals belonging to 16 species (Table 2). Other identified species were mainly forest and floodwater species such as *Aedes* (*Aedes*) *cinereus* s.l. (Meigen) (*n* = 415) (We use *Aedes cinereus* s.l. for *Ae. cinereus* Meigen and *Aedes geminus* Pneus considering individuals of both species can only be morphological separated with certainty based on the shape of male genitalia and given little is known on the medical importance of these sibling species, individuals were not further distinguished.), *Aedes* (Ochlerotatus) *annulipes* (Meigen) (*n* = 273), *Aedes* (Ochlerotatus) *rusticus* (Rossi) (*n* = 627), *Anopheles plumbeus* (Stephens) (*n* = 21), *Coquillettidia richiardii* (Ficalbi) (*n* = 36), and *Culex pipiens* L. (*n* = 251). *Ae. koreicus* was primarily collected from gravid traps (67%), the MMLP traps yielded only half as much individuals (33%) and none were sampled with the BG Sentinel traps. Both females (70%) and males (30%) of the species were collected; all collected female specimens were unfed. The first adult *Ae. koreicus* specimens were collected on 18 May 2009 from site 1 after which the population

Table 2. Mosquito species and numbers collected in 2008 (on site 1 and site 2 in May and Sept. with 1 MMLP) and 2009 (on site 1 and site 2 from April till Oct. with 2 MMLP, 2 Gravid Traps, and 2 BG Sentinel traps per location)

Genus name	Species name	2008	2009
Aedes	*Annulipes*	3	273
	Cantans	2	84
	Cinereus/geminus	35	415
	Communis	9	10
	Geniculatus	2	26
	Koreicus	6	69
	Punctor	6	44
	Rusticus	28	627
Anopheles	*Claviger*	1	18
	Maculipennis s.l.	3	4
	Plumbeus	2	21
Coquillettidia	*Richiardii*	0	36
Culex	*Pipiens*	14	251
	Torrentium	0	43
Culiseta	*Annulata*	19	4
	Morsitans	2	25

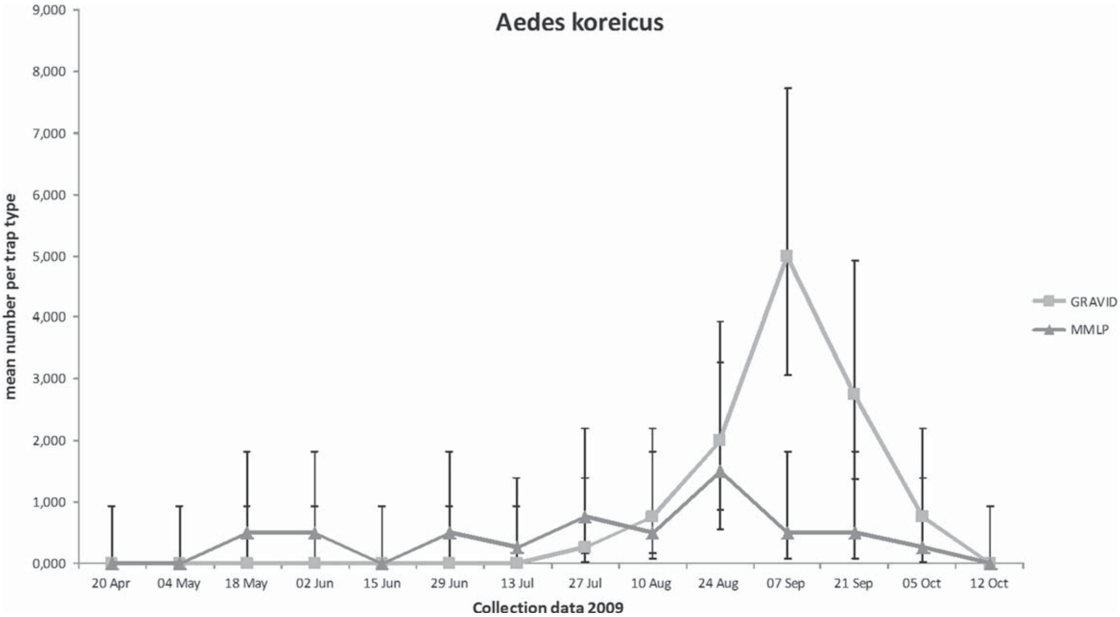

Fig. 1. Overall phenology of *Aedes koreicus* at Maasmechelen; Gravid = gravid traps representing female oviposition activity and larval eclosion; MMLP = Mosquito Magnet Liberty Plus traps representing adult activity.

did not increase in numbers until late summer. On site 2, the first specimens were trapped on 27 July 2009. Overall, the population steadily increased during summer and peaked during August to September (Fig. 1). The peak observed in the MMLP data represent adult activity, while the gravid trap data reflect the female oviposition activity. Adults of the species were still present on both sites until the beginning of October 2009. This was in contrast with the other observed *Aedes s*pecies that exhibit a peak in spring. Only *Cx. pipiens* was found frequently until the end of the sampling campaign in October 2009.

Ae. koreicus larvae collected in 2008 were mainly retrieved from artificial containers; only a few were sampled in natural oviposition habitats (Table 3). Immatures were found in two large (heavy vehicles) and several small abandoned tires and from a discarded and rusted water recipient. In natural habitats the species was retrieved from two water filled mud tracks up to 1.5 km from site 1. In 2009, 61 possible oviposition sites were screened, of which only 17 could be identified in natural habitats. All larvae of *Ae. koreicus* were retrieved solely from artificial recipients. Of 44 oviposition sites screened 11 were found positive. A high-larval density was observed in scrapped heavy construction equipment, filled with 5–10 cm of water, debris and colonized by some plants (Table 3). Other positive artificial containers included a plastic tray filled with water containing green algae and several tires (heavy equipment and cars) filled with organic rich water. All artificial containers positive with *Ae. koreicus* larvae were situated in a radius of 500 m around site 1 and none around site 2. Larvae of *Ae. koreicus* were detected from early May and were readily found during summer months until the beginning of October. Larvae of *Cx. pipiens* and *Culiseta annulata* (Schrank) were found together in the same containers in 2008 and 2009. Positive oviposition traps were found at two occasions (10 August and 7 September 2009) and only on one easterly path characterized as forest-urbanized area. The first positive oviposition trap for *Ae. koreicus* was one km away from site 1 while the last one was already four km away and close to an urbanized district.

Climatic Data Comparison. For some of the climatic variables, no or only a small and scattered area could be found where Belgium is similar to the island of Jeju-do. This was mainly the case for summer tem-

Table 3. Extrapolated no. of *Aedes koreicus* larvae per liter in positive oviposition sites per collection date and oviposition type

Year	Date	No. larvae/liter	Type of oviposition site
2008			
	5/07/2008	1	Temporary surface pool
	5/07/2008	20	Small tires (car)
	8/07/2008	20	Large tires (heavy equipment)
	3/08/2008	25	Small tires (car)
	3/08/2008	10	Temporary surface pool
	30/08/2008	15	Rusted water recipient
2009			
	6/05/2009	25	Old construction equipment
	6/05/2009	10	Small tires (car)
	6/05/2009	10	Small tires (car)
	6/05/2009	10	Small tires (car)
	6/05/2009	20	Large tires (heavy equipment)
	22/06/2009	25	Old construction equipment
	22/06/2009	25	Old construction equipment
	22/06/2009	20	Old construction equipment
	14/09/2009	20	Old construction equipment
	14/09/2009	30	Plastic tray
	14/09/2009	15	Small tires (car)

perature, humidity, and precipitation. However, the results for 26 out of 70 variables showed that the major part of Belgium features climatic conditions similar to Jeju-do (Table 1). Variables yielding a high similarity between Jeju-do and Belgium included winter temperatures, and the number of freezing days and nights.

Discussion

In 2008, the exotic mosquito species *Ae. koreicus,* until then restricted to temperate Asia, was collected in an area of eastern Belgium. Specimens were found during 2008 on different occasions, confirming the species presence on site. All Belgian specimens showed consistent morphological differences with the described specimens from the Korean peninsula but resembled more closely to specimens described from Jeju-do province, an island southern of the peninsula (Tanaka et al. 1979). This is the first record of the species outside its region of origin and evidence of its ability to colonize new territories. Subsequently, during spring 2011, *Ae. koreicus* was also reported from north–eastern Italy where the species was discovered in private gardens and in the cemetery of a small village in the Belluno province (ProMed 2011). This area is under routine *Ae. albopictus* monitoring because 2009 over which the species was not present or went undetected (Capelli et al. 2011).

Information on the species phenology and ecology in its native range are scarce and scattered, none of the consulted papers targeted exclusively *Ae. koreicus.* During this study, adults and immatures were found in an area of 6 km^2 although the population was focalized around the industrial zone of site 1. In 2009, the species was first found in early spring but reached its peak during late summer (early September) and larvae were present until at least 5 October 2009. In its region of origin *Ae. koreicus* appears during late spring (Ho 1931) and seems to reach peak activity during the summer months (Kim et al. 2006, 2007, 2009). The combined use of different trapping techniques offers advantages certainly if the species is not well known (and thus the efficiency of the used traps is uncertain). The MMLP reflects the activity of the adult species, while the gravid trap represent the female oviposition activity as the latter trap accidentally functioned as a large artificial oviposition site (because of falling leaves and twigs). In Belgium, larvae of the species have so far only been found in organic rich water, mainly in artificial containers like abandoned tires and scrapped construction equipment. High productivity was observed in the latter, probably because of the size of these machines. This oviposition type remained positive all season. Abandoned tires that were prospected included two large construction tires (e.g., from cranes) and several car tires. There was no notable difference observed in productivity correlated to the size of the tires. In 2008, larvae of the species were also observed in temporary muddy road tracks, these were also monitored in 2009 and 2010, but were continuously dried out. Overall, there was a low supply of (semi) natural oviposition sites in this region. These sites seem to play a negligible role because of their low availability and low productivity. The species appears to be adapted to artificial oviposition sites in Belgium. In its native range *Ae. koreicus* larvae are known to develop in a number of artificial sites such as garden ponds, water drums (Rueda et al. 2006), and other recipients (Ho 1931), as well in natural sites such as tree holes and stone cavities containing rain water and decaying tree leaves (Feng 1938). Nothing is known on the species local feeding habits, its competition potential and thus the effect on the native fauna. In Belgium, 15 other Culicidae species were collected on the sites where *Ae. koreicus* occurs. Most of these are early season species already disappearing on site when the population of *Ae. koreicus* increases. Competition probably occurs mainly between *Ae. koreicus* and co-occurring *Cs. annulata* and *Cx. pipiens,* both widespread species in Belgium.

In addition to the observations of the species presence in 2008 and 2009, larval and adult collections at three random visits during spring–summer 2010 confirmed the establishment of the species on site. For the moment, the species seems to maintain a viable hibernating population at the industrial zone of Maasmechelen from which the population temporarily and locally expand. It is possible that we are observing a lag period (the time period between the successful establishment of a species and the start of its invasive spread) in the species invasion process (Ellstrand & Schierenbeck 2000). Similar observations are made with another exotic mosquito (*Ae. j. japonicus*) present in Belgium. Studies addressing this phenomenon in Belgium are therefore highly recommended.

Biological invasion remains a multistep process, involving 1) introduction into a new area, 2) establishment, and 3) spread (Shigesada and Kawasaki 1997). It is often admitted that multiple introductions are required for the success of biological invasion; however, there are examples that even isolated founding populations with strong reduced genetic variation may still adapt very quickly (Dlugosch and Parker 2008). The probability of, establishment and invasion success of exotic species depends initially upon matching climatic conditions (Tatem et al. 2006, Tatem and Hay 2007). A comparison between climatic factors in Jeju-do and Belgium showed similar annual mean temperatures (9–11°C) and winter temperatures and freezing days and nights in four consecutive years (2004–2008); thus, allowing winter survival of the species. In contrast, the seasonal pattern of relative humidity and precipitation in Belgium deviated strongly from that of Jeju-do. Low humidity may be a limiting factor for establishment and expansion of a mosquito population (Mackenzie et al. 2000). However, an annual precipitation of 800 mm is recorded in Belgium, which is higher than the calculated yearly rainfall of 500 mm, needed to provide enough water to fill container oviposition sites (as calculated by Medlock et al. 2006). Though the route of importation of the species into Belgium could not be determined, it may have occurred via international trade as a large industrial center was located nearby.

These findings highlight once again the growing possibilities for exotic mosquitoes to spread to new areas and the consequent need for an European entomological surveillance system and monitoring of the main importation pathways of vectors. In particular, a better traceability of imported and exported goods at risk will improve the surveillance possibilities. Prompt and adequate control is needed after detecting an exotic species to eliminate it or at least to limit its further spread. The presence of this species was reported to the Belgian health authorities, however, up to now no actions on prevention, control, or monitoring were planned.

Ae. koreicus is currently established in the studied region, although there are no complaints of nuisance at the moment of writing nor any evidence of expansion to other areas of Belgium. The species was most probably introduced through human trade. The introduction of new potential disease vectors into Europe seems to be a result of suitable entrance points, created by intense worldwide trade and suitable environmental conditions.

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